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Antioxidant, anti-inflammatory and gastroprotective activity of *Filipendula ulmaria* (L.) Maxim. and *Filipendula vulgaris* Moench

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Abstract

Ethnopharmacological relevance

Meadowsweet (*Filipendula ulmaria* (L.) Maxim.) and dropwort (*Filipendula vulgaris* Moench) are herbaceous perennials employed in folk medicine for their antirheumatic, antipyretic and anti-ulcer properties.

Aim of the study

To assess ethnomedicinal claims through investigation of antioxidant, anti-inflammatory and gastroprotective effects of *F. ulmaria* and *F. vulgaris* lyophilized flower infusions (LFIs) as well as the *F. vulgaris* isolated flavonoids spiraeoside, kaempferol 4′-*O*-glucoside, astragalin 2′′-*O*-gallate, mixture of hyperoside 2′′-*O*-gallate and isoquercitrin 2′′-*O*-gallate, and a tannin tellimagrandin II.

Materials and methods

Free radical scavenging activity of the tested samples was determined by examining their ability to neutralize DPPH and OH radicals *in vitro*, whereas reducing properties were assessed in Ferric Reducing Antioxidant Power (FRAP) assay. Anti-inflammatory activity was studied *ex vivo* in human platelets by monitoring the effect on eicosanoid biosynthesis. Gastroprotective action was estimated in animal model of acute gastric injury induced by ethanol.

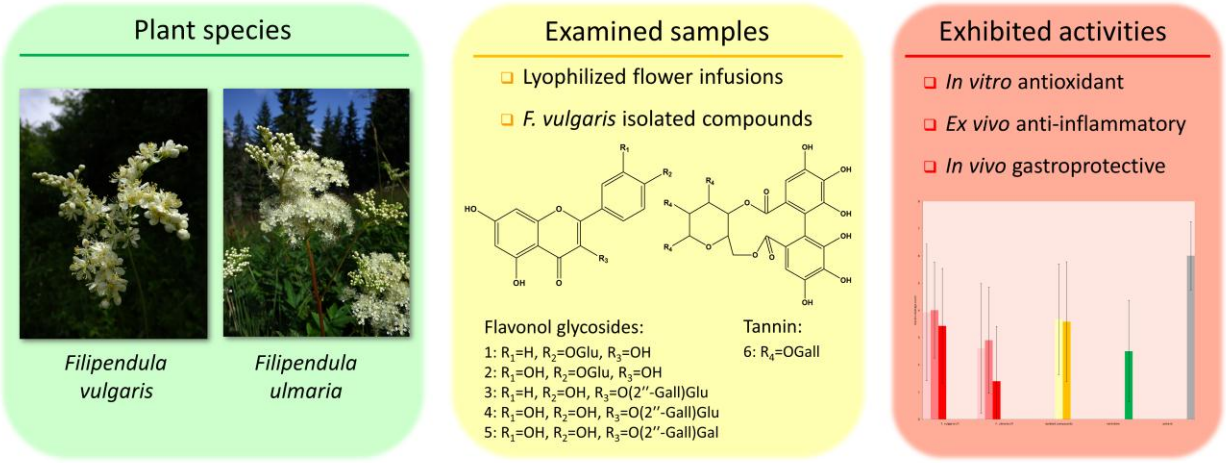
Results

LFIs and spiraeoside exerted activities comparable to those of positive control in DPPH-radical scavenging and FRAP antioxidant assays, whereas notable hydroxyl radical scavenging ability was demonstrated only for spiraeoside (IC_{50} =5.1 μg/mL). Among tested samples, astragalin 2″-*O*-gallate (IC_{50} =141.1 μg/mL) and spiraeoside (IC_{50} =4.69 μg/mL) the most markedly inhibited production of pro-inflammatory prostaglandin E_2 and 12(S)-hydroxy-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoic acid in human platelets, respectively. Examination of LFIs (100–300 mg/kg, p.o.) gastroprotective action in rats revealed their capacity to preserve mucosal integrity. In addition, spiraeoside (50 mg/kg, p.o.) and tellimagrandin II (40 mg/kg, p.o.) showed ulcer preventive ability.

Conclusion

Current study supports documented traditional use of investigated herbs and indicates that flavonoid and tannin components are partially responsible for the demonstrated pharmacological activities.

Graphical abstract



Keywords: *Filipendula*, meadowsweet, dropwort, antioxidant, anti-inflammatory, gastroprotective.

1. Introduction

Meadowsweet (*Filipendula ulmaria* (L.) Maxim., syn. *Spiraea ulmaria* L.; Rosaceae) and dropwort (*Filipendula vulgaris* Moench, syn. *Filipendula hexapetala* Gilib.; Rosaceae) are herbaceous perennial plants which can be found in Europe (Ball, 1968). Their flowers are employed in traditional medicine with reputed beneficial effects in various ailments, such as rheumatism, gout, common cold, fever, infections and peptic ulcer disease (HMPC, 2011a; Jarić et al., 2007; Šarić-Kundalić et al., 2011; Vogl et al., 2013). European Medicines Agency approved use of meadowsweet flowers, *Filipendulae ulmariae flos*, for preparation of the traditional herbal medicinal products (HMPC, 2011b).

Previous animal studies revealed that *Filipendula* species counteracted inflammatory processes and reduced pain (Katanić et al., 2016; Samardžić et al., 2016). A limited number of mechanistic *in vitro* experiments indicated that *Filipendula* extracts inhibited the classical pathway of the complement system (ESCOP, 2003), production of pro-inflammatory cytokines (Drummond et al., 2013) and expression of adhesion molecules (Vogl et al., 2013). Gastroprotection was to some extent explored earlier in meadowsweet (ESCOP, 2003), whereas respective data concerning dropwort are not available. However, scientific basis for folkloric applications of *Filipendula* species in the treatment of inflammatory conditions and peptic ulcer is not firmly established and additional investigations could considerably contribute to the estimation of reported use in traditional medicine.

Phytochemical analysis showed that *Filipendula* species contain significant amount of polyphenolics, among which flavonol glycosides and ellagitannins are the most prominent (Olennikov and Kruglova, 2013). Despite the quite thorough characterization of the corresponding herbal drugs, the active constituents are not sufficiently defined and their role in the medicinal effects of meadowsweet and dropwort flowers is still not fully known.

The current study was designed with the aim to provide scientific evidence that supports ethnomedicinal claims through the investigation of the relevant pharmacological properties of two *Filipendula* species and their active constituents. Anti-inflammatory activity of *F. vulgaris* isolated compounds and *F. vulgaris* and *F. ulmaria* lyophilized flower infusions (LFIs) was examined *ex vivo* in human platelets by monitoring the effects on eicosanoid production. Gastroprotective activity was tested using the ethanol-induced acute mucosal injury in rats and

antioxidant activity was investigated because free radicals scavenging and reducing properties may contribute to the tested biological effects.

2. Materials and methods

2.1. General

All solvents used were of analytical grade, except those employed for high pressure liquid chromatography which were of HPLC or LC-MS quality. Reagents and substances required for chemical analysis of LFIs and pharmacological activities investigation were purchased from Sigma-Aldrich (Germany and USA), Carl Roth (Germany), Acros (Belgium), Fluka (Switzerland) and Zdravlje Actavis (Serbia). Spiraeoside, used for HPLC analysis, was isolated from *Anthemis triumfetti* (L.) DC. (Pavlović et al., 2006). Platelets concentrates were obtained from the Institute for Blood Transfusion of Vojvodina (Novi Sad, Serbia) immediately after expiry date for human use.

Five flavonoids and one tannin were isolated from the *F. vulgaris* flowers by column chromatography (using silica gel and Sephadex LH-20 as adsorbents), C18 vacuum liquid chromatography, preparative TLC on cellulose-coated plates and RP-C18 semi-preparative HPLC. In order to elucidate their structures, UV, MS and NMR (^{1}H and ^{13}C) spectra were recorded. Detailed description of employed procedures and instruments is given in the Supplementary material.

2.2. Plant material and preparation of lyophilized flower infusions

Dropwort flowers were collected in May 2013 and 2014 near Loćika village (Central Serbia), whereas meadowsweet flowers were harvested at Mt. Kopaonik (Central Serbia) in July 2016. Plant material was identified by Professor Branislava Lakušić (Department of Botany, University of Belgrade — Faculty of Pharmacy) and voucher specimens (*F. vulgaris* voucher number: 3713HFF; *F. ulmaria* voucher number: 3872HFF) were deposited in the Herbarium of the Department of Botany, University of Belgrade — Faculty of Pharmacy.

Lyophilized flower infusions (LFIs) were obtained in accordance with traditional method of preparation. Namely, boiling water was poured over comminuted dried plant material (drug-

solvent 1:20), the mixture was allowed to steep for 30 minutes with occasional stirring and filtered. The resulting extracts were freeze-dried. LFIs were obtained as fine powders with 31.22% and 23.80% yield, for *F. vulgaris* and *F. ulmaria*, respectively.

2.3. HPLC analysis of *F. vulgaris* and *F. ulmaria* lyophilized flower infusions

Analyses of LFIs were performed on an Agilent 1100 Liquid chromatograph coupled to a DAD detector and equipped with Zorbax Eclipse XDB-C18 analytical column (4.6×250 mm, 5 μm, Agilent). The aqueous solutions of LFIs were filtered through a 0.45 μm membrane filter and manually injected (20 μL). Separation was achieved at 25 °C by using a mixture consisting of solvent A (H_3PO_4 in H_2O, pH 2.75) and solvent B (solvent A : acetonitrile, 10 : 90 v/v). Gradient composition of the binary mobile phase is presented in Table 1S (Supplementary material). LFIs constituents were identified by matching their retention times and UV spectra with the data of standards obtained under the same chromatographic conditions. Contents of detected substances were determined from calibration curves of gallic acid (y=202385x-293.76, R^2=0.9992, concentration range 0.008– 0.131 mg/mL), salicylic acid (y=318761x+84.572, R^2=0.9999, 0.002–0.066 mg/mL), hyperoside (y=23450x-20.696, R^2=0.9993, 0.006–0.090 mg/mL), astragalin (y=39970x, R^2=0.9999, 0.009–0.140 mg/mL), isoquercitrin (y=19482x-10.686, R^2=1, 0.01–0.66 mg/mL), spiraeoside (y=13213x+45.692, R^2=0.9979, 0.015–0.240 mg/mL) and ellagic acid (y=14063x+62.467, R^2=0.999, 0.008–0.130 mg/mL). For the purpose of quantification, detection was set at 210 nm for gallic acid and salicylic acid and at 350 nm for other compounds.

2.4. Antioxidant assays

In vitro antioxidant activity of spiraeoside and LFIs was determined using spectrophotometric methods as previously described (Kukić et al., 2006). Briefly, DPPH (2,2-diphenyl-1-picrylhydrazyl) and 2-deoxyribose assays were used to estimate free radical scavenging capacity of the tested samples. Results are expressed as IC_{50}, i.e. concentration of extract or compound causing 50% of DPPH or hydroxyl radical neutralization, respectively, and they were determined by regression analysis. FRAP (Ferric Reducing Antioxidant Power) assay, which is based on the ability of a sample to convert Fe^{3+}-2,4,6-tris-(2-pyridyl)-s-triazine (Fe^{3+}-

TPTZ) to Fe^{2+}-TPTZ complex, was applied to examine reducing properties. Results are expressed as FRAP value, i.e. mmol/g Fe^{2+} in the tested extract or compound.

2.5. Inhibition of eicosanoid biosynthesis

Anti-inflammatory activity of isolated flavonoids and LFIs was studied *ex vivo* in human platelets by monitoring the inhibition of synthesis of pro-inflammatory mediators prostaglandin E_2 (PGE_2), thromboxane B_2 (TXB_2) and 12(S)-hydroxy-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoic acid (12-HETE) (Lesjak et al., 2013). The formation of mediators was induced by addition of calcium ionophore A23187 and their concentrations were measured by LC-MS/MS. Inhibition of mediator production, I(%), was calculated according to the equation: $I(\%)=100\times(R_0-R)/R_0$. Response ratios (metabolite peak area/internal standard peak area) in the control reaction and tested samples were designated R_0 and R, respectively. The results were expressed as IC_{50} values (obtained in Origin software, version 8.0), i.e. concentration of extract or compound leading to 50% inhibition of mediator synthesis.

2.6. Gastroprotective activity

Investigation was conducted on male Wistar rats (6–8 weeks old; average weight 220 g) obtained from Military Academy Breeding Farm, Belgrade, Serbia. After delivery to our laboratory, animals were allowed to acclimatize for two weeks. Twenty hours before the start of the experiment, rats were fasted; meanwhile they had free access to tap water. Gastric lesions were induced by oral gavage of absolute ethanol (5 mL/kg, p.o.). Tested *F. ulmaria* and *F. vulgaris* LFIs (100–300 mg/kg, p.o.) and isolated compounds spiraeoside (50 mg/kg, p.o.) and tellimagrandin II (40 mg/kg, p.o.), were administered an hour before inducing the lesions. Animals from positive and negative control groups received ranitidine (20 mg/kg, p.o.) or vehicle (water, 1 mL/kg, p.o.), respectively. One hour after the treatment with absolute ethanol, animals were euthanized in atmosphere with high carbon dioxide content. Next, their stomachs were removed, opened along greater curvature and rinsed with saline solution. Lesions were examined under a magnifying glass (3×). Damage to mucosa was expressed as gastric damage score by using modified scoring system of Adami et al.: **0**, no lesions; **0.5**, slight hyperaemia or ≤5 petechiae; **1**, ≤5 erosions ≤5 mm length; **1.5**, ≤5 erosions ≤5 mm length and many petechiae;

2, 6–10 erosions ≤5 mm length; **2.5**, 1–5 erosions >5 mm length; **3**, >5–10 erosions >5 mm length; **3.5**, >10 erosions >5 mm length; **4**, 1–3 erosions ≤5 mm length and 0.5–1 mm width; **4.5**, 4–5 erosions ≤5 mm length and 0.5–1 mm width; **5**, 1–3 erosions >5 mm length and 0.5–1 mm width; **6**, 4–5 grade 5 lesions; **7**, ≥6 grade 5 lesions; **8**, complete lesion of the mucosa with hemorrhage. Presence of statistically significant differences (*, $p<0.05$; **, $p<0.01$) between test and control animal groups was estimated using Mann-Whitney *U*-test in SPSS software version 20.0 (Đorđević et al., 2012). Experiment was approved by the Institutional Animal Care and Use Committee of the University of Belgrade — Faculty of Pharmacy (the bioethical allowance number: 323-07-1193/2014-05). All procedures were in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of Europe.

3. Results and discussion

3.1. Structure elucidation of the *F. vulgaris* flowers isolated compounds

Processes of isolation, purification and structure elucidation led to the identification of five flavonol glycosides (**1**–**5**) and one ellagitannin (**6**) of the *F. vulgaris* flowers. UV and NMR (^{1}H, ^{13}C) spectra of the isolated compounds were in agreement with previous reports (Supplementary material) and MS data corresponded to the determined structures. Based on literature, compound **1** was identified as kaempferol 4′-*O*-*β*-D-glucoside; **2** as spiraeoside (quercetin 4′-*O*-*β*-D-glucoside); **3** as kaempferol 3-*O*-(2″-*O*-galloyl)-*β*-D-glucoside (astragalin 2″-*O*-gallate); mixture of **4** and **5** was identified as mixture of quercetin 3-*O*-(2″-*O*-galloyl)-*β*-D-glucoside (isoquercitrin 2″-*O*-gallate) and quercetin 3-*O*-(2″-*O*-galloyl)-*β*-D-galactoside (hyperoside 2″-*O*-gallate) (2:1 w/w, based on HPLC-UV chromatograms and NMR spectra integration), respectively; and finally, **6** was identified as tellimagrandin II (1,2,3-trigalloyl-4,6-hexahydroxydiphenoyl-*β*-D-glucopyranose). Presence of these constituents, with the exception of kaempferol 3-*O*-(2″-*O*-galloyl)-*β*-D-glucoside and quercetin 3-*O*-(2″-*O*-galloyl)-*β*-D-glucoside, was demonstrated earlier in *F. vulgaris* (Olennikov and Kruglova, 2013; Pukalskienė et al., 2015). A recent mass spectral analysis of dropwort extract showed presence of kaempferol *O*-galloyl-hexoside, consistent with our results (Pukalskienė et al., 2015).

Compounds **1**, **2** and **3** and mixture **4**+**5** were tested for their anti-inflammatory activity, whereas the gastroprotective effects were investigated for the compounds **2** and **6**. The antioxidant activity of compound **2** was also assayed.

3.2. Composition of lyophilized flower infusions determined by HPLC

HPLC analysis of LFIs revealed the presence of flavonol glycosides, phenolic acids and tannin (Table 1). Both LFIs contained glycosides of quercetin and kaempferol, but with certain differences in the composition. The dominant flavonoid constituent in both samples was spiraeoside (55.67±1.82 and 46.17±1.67 mg/g in *F. vulgaris* and *F. ulmaria* LFIs, respectively). In addition, the presence of isoquercitrin, hyperoside, astragalin, isoquercitrin 2″-*O*-gallate, hyperoside 2″-*O*-gallate, astragalin 2″-*O*-gallate and kaempferol 4′-*O*-*β*-D-glucoside was unambiguously confirmed in *F. vulgaris* LFI. Beside the dominant spiraeoside, meadowsweet LFI contained miquelianin (quercetin 3-*O*-*β*-D-glucuronide), kaempferol 4′-*O*-*β*-D-glucoside and a kaempferol glycoside. However, in contrast to dropwort LFI, hyperoside, isoquercitrin, astragalin and their respective 2″-*O*-galloylated derivatives were not detected in the meadowseet LFI.

Regarding phenolic acids, both LFIs contained gallic acid as the most abundant, 10.22±0.28 mg/g of dropwort LFI, 11.15±0.13 mg/g of meadowsweet LFI. Salicylic acid was present in lower amounts in both samples, whereas ellagic acid was detected only in dropwort LFI.

Tellimagrandin II, a hydrolysable tannin, occurred in substantial amounts in investigated extracts of dropwort (15.80±0.46 mg/g) and meadowsweet (11.48±0.13 mg/g).

Our results correspond relatively well with the recent studies of *F. vulgaris* and *F. ulmaria* flowers constituents (Bączek et al., 2012; Gniewosz et al., 2014; Olennikov and Kruglova, 2013).

Table 1. Composition of *F. ulmaria* and *F. vulgaris* lyophilized flower infusions (LFIs).

Compound	Content (mg/g)	
	F. ulmaria LFI	*F. vulgaris* LFI
Flavonol glycosides		

Spiraeoside	46.17±1.67	55.67±1.82
Hyperoside	nd	5.12±0.17
Hyperoside 2″-*O*-gallate	nd	3.8±0.1[a]
Isoquercitrin	nd	13.24±0.33
Isoquercitrin 2″-*O*-gallate	nd	6.3±0.19[b]
Miquelianin	26.95±0.27[b]	nd
Astragalin	nd	11.22±0.35
Astragalin 2″-*O*-gallate	nd	4.58±0.01[c]
Kaempferol 4′-*O*-*β*-D-glucoside	8.27±0.12[c]	4.91±0.03[c]
Kaempferol glycoside	7.37±0.33[c]	nd
Phenolic acids		
Gallic acid	11.15±0.13	10.22±0.28
Ellagic acid	nd	3.8±0.18
Salicylic acid	1.55±0.03	3.5±0.12
Tannin		
Tellimagrandin II	11.48±0.13[d]	15.80±0.46[d]

Data are presented as means±SD, *n*=3; nd, not detected; [a] calculated as hyperoside; [b] calculated as isoquercitrin; [c] calculated as astragalin; [d] calculated as gallic acid.

3.3. Antioxidant activity

In DPPH and FRAP assays, LFIs and their quantitatively dominant flavonoid glycoside spiraeoside displayed significant antioxidant properties comparable to those of the control substance (Table 2). Spiraeoside surprisingly exhibited lower potency than LFIs in the DPPH assay, possibly due to the lack of a key structural feature (*o*-dihydroxy system) generally required for high flavonoid antiradical effectiveness (Yokozawa et al., 1998).

On the other hand, hydroxyl radical scavenging of LFIs, determined by 2-deoxyribose assay, did not reach 50% inhibition. However, spiraeoside was active (IC_{50}=5.1 μg/mL).

Table 2. Antioxidant activity of lyophilized flower infusions (LFIs) and spiraeoside.

	DPPH IC_{50}[a] (μg/mL)	$^{\bullet}OH$ IC_{50}[b] (μg/mL)	FRAP value[a] (mmol Fe^{2+}/g)
F. vulgaris LFI	9.10±0.09	ne	7.72±0.04
F. ulmaria LFI	8.45±0.06	ne	4.46±0.1
Spiraeoside	19.54±0.23	5.1	15.02±0.64
L-ascorbic acid	4.09[c]	nt	13.29±0.26
Quercetin	nt	3.1[c]	nt

nt, not tested; ne, not effective – IC_{50}>16.67 μg/mL; [a] expressed as mean±SD (*n*=3); [b] data from three independent experiments; [c] Kukić et al., 2006.

Demonstrated good anti-DPPH and Fe^{3+} reducing properties of LFIs are in agreement with previously published reports on different types of *Filipendula* extracts (Maksimović et al., 2007; Olennikov et al., 2016). Based on the results of HPLC analysis (Table 1), it could be assumed that LFIs considerable antioxidant capacity is related to their high content of polyphenolic compounds. Moreover, it seems likely that spiraeoside participates in the LFIs total antioxidant effect. Antioxidants could assist in attenuation of inflammatory response and in prevention of gastric ulceration (Đorđević et al., 2012), so the data obtained in our experiment are of relevance for the investigated medicinal plants.

3.4. Inhibition of eicosanoid biosynthesis *ex vivo*

In this study, hypothesis that diminished eicosanoid formation is involved in anti-inflammatory effect of flowers of investigated *Filipendula* species was checked using stimulated human platelets as an *ex vivo* model system. After their exposure to LFIs or *F. vulgaris* isolated compounds, the concentrations of generated eicosanoids (PGE_2, TXB_2 and 12-HETE) were measured. Most of the tested samples, i.e. LFIs and isolated flavonoids, in the applied concentration ranges (0.25–10 mg/mL for LFIs; 0.4–200 μg/mL for kaempferol 4′-*O*-*β*-D-glucoside, astragalin 2″-*O*-gallate and mixture of isoquercitrin 2″-*O*-gallate and hyperoside 2″-*O*-gallate; 2–160 μg/mL for spiraeoside) were able to decrease the levels of monitored metabolites (Table 3).

IC_{50} values were in the range of 0.961–4.401 mg/mL and 3.415–6.768 mg/mL for dropwort and meadowsweet LFIs, respectively, and were higher than the corresponding values of control substances (acetylsalicylic acid and quercetin). *F. vulgaris* LFI more effectively inhibited production of all monitored mediators compared to *F. ulmaria* LFI. Investigated extracts exhibited the greatest potency in attenuation of 12-HETE synthesis, weaker activity was observed for reduction of PGE_2 level, whereas only *F. vulgaris* LFI was able to reach more than 50% inhibition of TXB_2 generation.

Capacity of isolated flavonoids to affect concentration of eicosanoids varied considerably. Tested compounds did not reach 50% suppression of TXB_2 formation. Regarding inhibition of PGE_2 production, astragalin 2″-*O*-gallate was the most effective among examined compounds (IC_{50}=141.1 μg/mL). Mixture of hyperoside 2″-*O*-gallate and isoquercitrin 2″-*O*-gallate (1:2), as well as spiraeoside, decreased PGE_2 levels by more than 50%, but the effect was not concentration-dependent. Spiraeoside inhibited production of 12-HETE with notable potency (IC_{50}=4.69 μg/mL) comparable to that of quercetin (IC_{50}=7.44 μg/mL). Astragalin 2″-*O*-gallate and mixture of hyperoside 2″-*O*-gallate and isoquercitrin 2″-*O*-gallate were less active with IC_{50} values of 90.21 and 121.8 μg/mL, respectively. It appears that glycosylation of quercetin hydroxyl group at the position 4′ does not diminish potential to inhibit 12-HETE production. On the other hand, galloylated glycosyl groups at the position 3 of aglycone and/or lack of hydroxyl group at the position 3′ seems to be unfavorable. Kaempferol 4′-*O*-*β*-D-glucoside displayed no activity in the tested concentration range.

Table 3. Inhibition of eicosanoid production by lyophilized flower infusions (LFIs) and *F. vulgaris* isolated compounds.

	12-HETE	**PGE_2**	**TXB_2**
	IC_{50} (μg/mL)		
LFIs			
F. vulgaris LFI	961±83	4241±67	4401±62
F. ulmaria LFI	3415±294	6768±108	ne
***F. vulgaris* isolated compounds**			
Kaempferol 4′-*O*-*β*-D-glucoside	na	na	na
Spiraeoside	4.69±0.4	ncd	ne
Astragalin 2″-*O*-gallate	90.21±7.76	141.1±2.24	ne

Mixture of hyperoside 2″-*O*-gallate and isoquercitrin 2″-*O*-gallate (1:2 w/w)	121.8±10.47	ncd	ne
Control substances			
Acetylsalicylic acid	na	5.58±0.53[a]	4.98±0.06[a]
Quercetin	7.44±0.65[a]	12.75±0.26[a]	53.69±2.47[a]

Results are expressed as means±SD (*n*=3); nt, not tested; na, not active — did not exhibit any activity; ne, not effective — did not reach 50 % inhibition; ncd, inhibition above 50% but not concentration dependent; [a] Lesjak et al., 2013.

Elevated eicosanoids production often accompanies inflammatory processes and suppression of their biosynthesis represents a clinically useful therapeutic approach. They are derived by enzymatically catalyzed transformation of arachidonic acid. PGE_2 plays many important roles in human organism, however, its vasodilating, hyperalgesic and pyrogenic properties are the most relevant for the current study (Rang et al., 2012). 12-HETE was identified as a neutrophil chemotaxin. In addition, involvement of this eicosanoid in different conditions (i.e. cancer and hypertension) is increasingly recognized (Porro et al., 2014). Thromboxane A_2 (TXA_2) exerts procoagulatory and proinflammatory actions (Rang et al., 2012; Semple et al., 2011). The effect of studied samples on TXA_2 biosynthesis was monitored indirectly, through the measurement of the concentration of its inactive metabolite (TXB_2). Direct determination of TXA_2 is not suitable as it rapidly and spontaneously converts to TXB_2 (Rang et al., 2012).

Taking into account the abovementioned facts and our results, it could be assumed that examined compounds contribute to the medicinal action of the *Filipendula* flowers. In that regard, current finding that spiraeoside, astragalin 2″-*O*-gallate and tested mixture of galloylated flavonoids attenuated synthesis of PGE_2 may be important to explain traditional use for alleviation of inflammatory and painful conditions. High potential of spiraeoside to suppress 12-HETE production represents considerable therapeutic potential and requires more detailed investigation. The observed low effectiveness of LFIs and isolated flavonoids in the inhibition of TXB_2 formation may indicate that studied samples do not modulate inflammation and coagulation processes mediated by TXA_2. *In vivo* studies are required to determine whether the pharmacokinetic fate influences the demonstrated activities of the tested flavonoids.

LFIs were characterized by high polyphenol content (Table 1) and prominent antioxidant properties (Table 2). Therefore, their mode of action may be based in part on neutralizing free

radicals, which are important mediators in the eicosanoid biosynthetic pathways (Schneider et al., 2007). Isolated compounds may also act in similar manner due to generally good scavenging ability of polyphenols (Lesjak et al., 2013).

Gallic and salicylic acid, that are also present in the LFIs (Table 1), were reported to inhibit COX-1 (one of key enzymes responsible for prostaglandins generation) (Chandramohan Reddy et al., 2010; Grosser et al., 2011). In addition to tested compounds, they may contribute to the LFIs effect on PGE_2 production. Hence, it seems that several different active constituents contribute to the beneficial anti-inflammatory effect of investigated LFIs.

In previous *in vitro* studies, which were carried out by using isolated cyclooxygenase enzymes or bovine seminal vesicle microsomes, several extracts of *F. ulmaria* inhibited PGE_2 biosynthesis (ESCOP, 2003; Katanić et al., 2016). Investigation of *F. vulgaris* effect on 12-HETE, TXA_2 and PGE_2 formation has not been conducted so far. Pain relieving and antiedematous properties of certain meadowsweet and dropwort extracts (Katanić et al., 2016; Samardžić et al., 2016) were demonstrated in animal studies, nevertheless, their mechanism of action and active constituents are still not sufficiently defined. Moreover, data regarding the traditionally used preparations lack.

The present experiment was carried out on the intact cells, enabling better prediction of the *in vivo* action when compared with the tests performed on the isolated enzymes or subcellular structures. More complete picture of the anti-inflammatory effect of tested samples was obtained, by detecting their influence on 12-HETE and TXA_2 in addition to PGE_2. The present study revealed the significant anti-inflammatory activity of flower infusions, preparation relevant for the reported ethnopharmacological application (HMPC, 2011b). Moreover, for the first time it was shown that flavonoids from *Filipendula* LFIs represented the active principles responsible for the suppression of the eicosanoids production.

3.5. Gastroprotective activity

F. vulgaris and *F. ulmaria* LFIs gastroprotective effects were demonstrated for all tested doses ($p<0.05$), with the exception of the lowest dose of *F. vulgairs* LFI (100 mg/kg) (Table 4). Average gastric damage scores (GDSs) in animal groups treated with dropwort and meadowsweet LFIs were in the range of 3.4–4 and 1.4–2.9, respectively. Although GDSs of LFIs

were the most reduced in animal groups receiving the highest quantities of the examined extracts (300 mg/kg), statistically significant dose-response relationship was not observed. Oral administration of isolated compounds, spiraeoside (50 mg/kg, GDS 3.6, $p<0.05$) and tellimagrandin II (40 mg/kg, GDS 3.7, $p<0.05$), also decreased severity of the formed lesions in the present animal study. Ranitidine, as a positive control, evidently protected gastric mucosa (GDS 2.5, $p<0.01$), whereas control group of animals which received vehicle (water) developed the most extensive damage of stomach mucosa (GDS 6).

Table 4. Average gastric damage scores (GDSs) in animal groups treated with lyophilized flower infusions (LFIs), isolated compounds, referent drug or vehicle.

		GDS
Lyophilized flower infusions		
F. vulgaris	100 mg/kg	3.92±2.5
	200 mg/kg	4±1.76*
	300 mg/kg	3.42±2.11*
F. ulmaria	100 mg/kg	2.6±2.38*
	200 mg/kg	2.9±1.95*
	300 mg/kg	1.4±2.01*
***F. vulgaris* isolated compounds**		
Tellimagrandin II 40 mg/kg		3.67±2.04*
Spiraeoside 50 mg/kg		3.58±2.2*
Referent drug		
Ranitidine 20 mg/kg		2.5±1.87**
Control		
Vehicle (water)[a]		6±1.26

Data are presented as means±SD (n=5–6); *, statistically significant versus control group for $p< 0.05$, and **, statistically significant versus control group for $p< 0.01$; [a] applied in the same volume as tested samples (1 mL/kg).

To the best of our knowledge, this is the first report of dropwort capability to prevent ethanol-induced mucosal injury. Established activity of LFI is in accordance with the literature data regarding meadowsweet preparations. Specifically, it was shown that *F. ulmaria* flowers decoctions were effective in some animal models of stomach ulcerations (ESCOP, 2003).

Demonstrated protective activity of isolated tannin tellimagrandin II is supported by the fact that molecules with relative structural resemblance applied in similar doses (pedunculagin, 50 mg/kg, i.g.; corilagin, 30 mg/kg, p.o.) exerted activity in the mice treated with the same harmful agent as in the current experiment (de Jesus et al., 2012; Klein-Júnior et al., 2017). Spiraeoside effectiveness is in line with previously observed capacity of some flavonoid glycosides to prevent stomach injury in animals (de Lira Mota et al., 2009).

Free radical species are involved in the onset of the mucosal damage evoked by necrotizing agent ethanol (Đorđević et al., 2012). Hence, pronounced antioxidant activity of tested LFIs and isolated compounds (Table 2) (Chen et al., 2014) probably contributed to the preservation of the stomach mucosal integrity. Furthermore, attenuation of lesions formation achieved by tellimagrandin II could be related to the recently demonstrated histidine decarboxylase inhibition *in vitro* (Nitta et al., 2013).

Our data strongly suggest that LFIs beneficial effects are associated with the presence of spiraeoside and tellimagrandin II, but role of other LFIs components should also be investigated in the future studies.

4. Conclusion

Lyophilized flower infusions of *F. vulgaris* and *F. ulmaria* were rich in polyphenolics that belong to the classes of flavonol glycosides, phenolic acids and hydrolysable tannins. These preparations, as well as the *F. vulgaris* flowers flavonoids (spiraeoside, astragalin 2″-*O*-gallate and mixture of hyperoside 2″-*O*-gallate and isoquercitrin 2″-*O*-gallate), were able to decrease production of proinflammatory eicosanoids *ex vivo* in human platelets. The investigated extracts,

along with spiraeoside and tellimagrandine II, also protected stomach mucosa of the rats from toxic effects induced by absolute ethanol.

The results obtained in this study support reported traditional use of meadowsweet and dropwort flowers in the treatment of inflammatory conditions and peptic ulcer and indicate that examined flavonoid and tannin compounds are pharmacologically active constituents.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

SS performed all the experiments (as a part of his PhD work) and wrote the manuscript. SS and JA performed HPLC analysis, isolation of compounds and interpretation of the collected data. SS, DB and MM conducted investigation of gastroprotective activity and analysis of the obtained results. SS and VT interpreted the recorded spectra and determined structures of the isolated compounds. SS and ZM designed experiments and interpreted the acquired data. All authors read and approved the final manuscript.

References

Bączek, K., Cygan, M., Przybył, J.L., Kosakowska, O., Węglarz, Z., 2012. Seasonal variation of phenolics content in above- and underground organs of dropwort (*Filipendula vulgaris* Moench). Herba Pol. 58, 24–32.

Ball, P.W., 1968. Filipendula Miller, in: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), Flora Europaea Vol 2. Roseaceae to Umbelliferae. Cambridge University Press, London, pp. 6–7.

Chandramohan Reddy, T., Aparoy, P., Kishore Babu, N., Anil Kumar, K., Kumar Kalangi, S., Reddanna, P., 2010. Kinetics and Docking Studies of a COX-2 Inhibitor Isolated from *Terminalia bellerica* Fruits. Protein Pept. Lett. 17, 1251–1257.

Chen, Y., Wang, J., Ou, Y., Chen, H., Xiao, S., Liu, G., Cao, Y., Huang, Q., 2014. Cellular antioxidant activities of polyphenols isolated from Eucalyptus leaves (*Eucalyptus grandis* × *Eucalyptus urophylla* GL9). J. Funct. Foods 7, 737–745.

de Jesus, N.Z.T., Falcão, H. de S., Gomes, I.F., Leite, T.J. de A., Lima, G.R. de M., Barbosa-Filho, J.M., Tavares, J.F., Silva, M.S. da, Athayde-Filho, P.F. de, Batista, L.M., 2012. Tannins, Peptic Ulcers and Related Mechanisms. Int. J. Mol. Sci. 13, 3203–3228.

de Lira Mota, K.S., Dias, G.E.N., Pinto, M.E.F., Luiz-Ferreira, Â., Monteiro Souza-Brito, A.R., Hiruma-Lima, C.A., Barbosa-Filho, J.M., Batista, L.M., 2009. Flavonoids with Gastroprotective Activity. Molecules 14, 979–1012.

Đorđević, S., Tadić, V., Petrović, S., Kukić-Marković, J., Dobrić, S., Milenković, M., Hadžifejzović, N., 2012. Bioactivity assays on *Carlina acaulis* and *C. acanthifolia* root and herb extracts. Dig. J. Nanomater. Biostructures 7, 1213–1222.

Drummond, E.M., Harbourne, N., Marete, E., Martyn, D., Jacquier, J., O'Riordan, D., Gibney, E.R., 2013. Inhibition of Proinflammatory Biomarkers in THP1 Macrophages by Polyphenols Derived From Chamomile, Meadowsweet and Willow bark. Phytother. Res. 27, 588–594.

ESCOP (European Scientific Cooperative on Phytotherapy), 2003. ESCOP Monographs: The Scientific Foundation for Herbal Medicinal Products, 2nd ed. Thieme New York, New York.

Gniewosz, M., Synowiec, A., Kraśniewska, K., Przybył, J.L., Bączek, K., Węglarz, Z., 2014. The antimicrobial activity of pullulan film incorporated with meadowsweet flower extracts (*Filipendulae ulmariae flos*) on postharvest quality of apples. Food Control 37, 351–361.

Grosser, T., Smith, F.M., FitzGerald, G.A., 2011. Anti-inflammatory, Antipyretic and Analgesic Agents; Pharmacotherapy of Gout, in: Brunton, L.L., Chabner, B.A., Knollmann, B.C. (Eds.), Goodman & Gilman's The Pharmacological Basis of Therapeutics. McGraw-Hill Medical Publishing Division, New York, pp. 959–1004.

HMPC (Committee on herbal medicinal products), 2011a. Assessment report on *Filipendula ulmaria* (L.) Maxim., herba and *Filipendula ulmaria* (L.) Maxim., flos. Reference number: EMA/HMPC/434892/2010. European Medicines Agency, London.

HMPC (Committee on herbal medicinal products), 2011b. Community herbal monograph on *Filipendula ulmaria* (L.) Maxim., flos. Reference number: EMA/HMPC/434894/2010. European Medicines Agency, London.

Jarić, S., Popović, Z., Mačukanović-Jocić, M., Djurdjević, L., Mijatović, M., Karadžić, B., Mitrović, M., Pavlović, P., 2007. An ethnobotanical study on the usage of wild medicinal herbs from Kopaonik Mountain (Central Serbia). J. Ethnopharmacol. 111, 160–175.

Katanić, J., Boroja, T., Mihailović, V., Nikles, S., Pan, S.-P., Rosić, G., Selaković, D., Joksimović, J., Mitrović, S., Bauer, R., 2016. *In vitro* and *in vivo* assessment of meadowsweet (*Filipendula ulmaria*) as anti-inflammatory agent. J. Ethnopharmacol. 193, 627–636.

Klein-Júnior, L.C., Silva, L.M. da, Boeing, T., Somensi, L.B., Beber, A.P., Rocha, J.A.R., Henriques, A.T., Andrade, S.F., Cechinel-Filho, V., 2017. The Protective Potential of *Phyllanthus niruri* and Corilagin on Gastric Lesions Induced in Rodents by Different Harmful Agents. Planta Med. 83, 30–39.

Kukić, J., Petrović, S., Niketić, M., 2006. Antioxidant Activity of Four Endemic *Stachys* Taxa. Biol. Pharm. Bull. 29, 725–729.

Lesjak, M.M., Beara, I.N., Orčić, D.Z., Ristić, J.D., Anačkov, G.T., Božin, B.N., Mimica-Dukić, N.M., 2013. Chemical characterisation and biological effects of *Juniperus foetidissima* Willd. 1806. LWT - Food Sci. Technol. 53, 530–539.

Maksimović, Z., Petrović, S., Pavlović, M., Kovačević, N., Kukić, J., 2007. Antioxidant activity of *Filipendula hexapetala* flowers. Fitoterapia 78, 265–267.

Nitta, Y., Kikuzaki, H., Azuma, T., Ye, Y., Sakaue, M., Higuchi, Y., Komori, H., Ueno, H., 2013. Inhibitory activity of *Filipendula ulmaria* constituents on recombinant human histidine decarboxylase. Food Chem. 138, 1551–1556.

Olennikov, D.N., Kashchenko, N.I., Chirikova, N.K., 2016. Meadowsweet Teas as New Functional Beverages: Comparative Analysis of Nutrients, Phytochemicals and Biological Effects of Four *Filipendula* Species. Molecules 22, 16.

Olennikov, D.N., Kruglova, M.Y., 2013. A new quercetin glycoside and other phenolic compounds from the genus *Filipendula*. Chem. Nat. Compd. 49, 610–616.

Pavlović, M., Kovačević, N., Couladis, M., Tzakou, O., 2006. Phenolic constituents of *Anthemis triumfetti* (L.) DC. Biochem. Syst. Ecol. 34, 449–452.

Porro, B., Songia, P., Squellerio, I., Tremoli, E., Cavalca, V., 2014. Analysis, physiological and clinical significance of 12-HETE: A neglected platelet-derived 12-lipoxygenase product. J. Chromatogr. B 964, 26–40.

Pukalskienė, M., Venskutonis, P.R., Pukalskas, A., 2015. Phytochemical composition and antioxidant properties of *Filipendula vulgaris* as a source of healthy functional ingredients. J. Funct. Foods 15, 233–242.

Rang, H.P., Dale, M.M., Ritter, J.M., Flower, R.J., Henderson, G., 2012. Rang & Dale's pharmacology, 7th ed. Churchill Livingstone, Edinburgh.

Samardžić, S., Tomić, M., Pecikoza, U., Stepanović-Petrović, R., Maksimović, Z., 2016. Antihyperalgesic activity of *Filipendula ulmaria* (L.) Maxim. and *Filipendula vulgaris* Moench in a rat model of inflammation. J. Ethnopharmacol. 193, 652–656.

Šarić-Kundalić, B., Dobeš, C., Klatte-Asselmeyer, V., Saukel, J., 2011. Ethnobotanical survey of traditionally used plants in human therapy of east, north and north-east Bosnia and Herzegovina. J. Ethnopharmacol. 133, 1051–1076.

Schneider, C., Pratt, D.A., Porter, N.A., Brash, A.R., 2007. Control of Oxygenation in Lipoxygenase and Cyclooxygenase Catalysis. Chem. Biol. 14, 473–488.

Semple, J.W., Italiano, J.E., Freedman, J., 2011. Platelets and the immune continuum. Nat. Rev. Immunol. 11, 264–274.

Vogl, S., Picker, P., Mihaly-Bison, J., Fakhrudin, N., Atanasov, A.G., Heiss, E.H., Wawrosch, C., Reznicek, G., Dirsch, V.M., Saukel, J., Kopp, B., 2013. Ethnopharmacological *in*

vitro studies on Austria's folk medicine—An unexplored lore *in vitro* anti-inflammatory activities of 71 Austrian traditional herbal drugs. J. Ethnopharmacol. 149, 750–771.

Yokozawa, T., Chen, C.P., Dong, E., Tanaka, T., Nonaka, G.-I., Nishioka, I., 1998. Study on the Inhibitory Effect of Tannins and Flavonoids against the 1,1-Diphenyl-2-picrylhydrazyl Radical. Biochem. Pharmacol. 56, 213–222.